

AMENDMENT OF THE CLAIMS:

This listing of claims will replace all prior versions, and listings, of claims in the application:

LISTING OF CLAIMS:

Claims 1-23 (cancelled).

Claim 24 (new): A method for producing a protected peptide fragment containing one or more modified amino acids or non-amino acids, which comprises: (a) preparing, on a weak acid-cleavable resin, a peptide main chain sequence of a peptide fragment which has a desired sequence comprising amino acids and/or non-amino acids, wherein at least one amino acid or non-amino acid is modified as represented by the formula -A(R)- (wherein A represents an amino acid or a non-amino acid, and R represents a substituent bound to a side chain of A which is introduced for modification), and wherein one or more reactive functional groups in a side chain of an amino acid or a non-amino acid which may cause an undesirable side reaction during preparation of a peptide fragment are protected with a protecting group, the one or more reactive functional groups being selected from the group consisting of a hydroxy group, an amino group, a guanidino group, an imidazolyl group, an indolyl group, a mercapto group and a carboxyl group, in a side chain of an amino acid or a non-amino acid, (b) deprotecting the protecting group of at least one reactive functional group in the side chain of an amino acid or a non-amino acid A which is to be modified with a substituent R without cleaving the peptide fragment from the weak acid cleavable resin, (c) modifying the deprotected side chain with a substituent R, and (d) cleaving the peptide fragment from the weak acid-cleavable resin under weakly acidic conditions without elimination of the protecting group in the peptide fragment.

Claim 25 (new): The method for producing a peptide fragment according to claim 24, wherein a protecting group for a reactive functional group in a side chain of an amino acid or a non-amino acid A which is to be modified with substituent R is a silyl protecting group, and a quaternary ammonium fluoride is used for eliminating the protecting group.

Claim 26 (new): The method for producing a peptide fragment according to claim 25, wherein the silyl protecting group is t-butyldimethylsilyl (TBDMS), t-butyldiphenylsilyl (TBDPS), triisopropylsilyl (TIPS), triisobutylsilyl (TIBS), t-hexyldimethylsilyl (ThxDMS) or triphenylsilyl (TPS), and the quaternary ammonium fluoride is tetrabutylammonium fluoride (TBAF), tetraethylammonium fluoride (TEF) or ammonium fluoride.

Claim 27 (new): The method for producing a peptide fragment according to any one of claims 24 to 26, wherein A is serine, threonine, cysteine, homocysteine, lysine, ornithine, glutamic acid, 2-aminoadipic acid, diaminoacetic acid, 2-aminomalonic acid, aspartic acid, tyrosine or asparagine, and R is bound to a reactive substituent in the side chain of A via an ester bond, an ether bond, a thioether bond, a disulfide bond, an amide bond, an O-glycoside bond or an N-glycoside bond.

Claim 28 (new): The method for producing a peptide fragment according to claim 27, wherein A is serine or threonine, and R is bound to the hydroxy group in the side chain of A via an ester bond.

Claim 29 (new): The method for producing a peptide fragment according to claim 28, wherein the peptide fragment is ghrelin or a derivative thereof, or a peptide fragment containing a modified amino acid in the ghrelin or a derivative thereof.

Claim 30 (new): A method for producing a modified peptide or protein, which comprises (a) preparing a protected peptide fragment containing one or more modified amino acids or non-amino acids by the method described in claim 24, (b) preparing a peptide fragment containing no modified amino acid or non-amino acid, and in which one or more reactive functional groups which may cause an undesirable side reaction, selected from the group consisting of a hydroxy group, an amino group, a guanidino group, an imidazolyl group, an indolyl group, a mercapto group and a carboxyl group, in the side chain of an amino acid or a non-amino acid, are protected, besides the peptide fragment of the (a), and condensing peptide fragments prepared in the (a) and the (b).

Claim 31 (new): The method for producing a modified peptide or protein according to claim 30, wherein condensation of the peptide fragments is performed by using a condensing agent.

Claim 32 (new): The method for producing a modified peptide or protein according to claim 31, wherein the condensing agent is 2-(1-hydrobenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), 2-(1-hydrobenzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), diphenylphosphorylazide (DPPA), diphenylphosphorocyanidate (DEPC), diisopropylcarbodiimide (DIPC), dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC).

Claim 33 (new): The method for producing a modified peptide or protein according to claim 31, wherein the condensing agent is diisopropylcarbodiimide (DIPC), dicyclohexylcarbodiimide (DCC) or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), and condensation of the peptide fragments (a) and (b) using the condensing agent is performed in the presence of 1-hydroxybenzotriazole (HOBt), 1-hydroxysuccinimide (HOSu) or 3,4-dihydro-3-hydroxy-4-oxo-benzotriazine (HOObt).

Claim 34 (new): The method for producing a modified peptide or protein according to any one of claims 30 to 33, which comprises producing a protected peptide fragment containing no modified amino acid or non-amino acid, by an enzymatic method or/and a genetic recombination method.

Claim 35 (new): The method for producing a modified peptide or protein according to claim 34, wherein the protected peptide fragment containing no modified amino acid or non-amino acid is produced by a method comprising;

step (1); a step of culturing a cell transformed with an expression vector having one of a nucleotide sequence encoding a peptide having an amino acid sequence of the peptide fragment (hereinafter referred to as desired peptide, in the present claim 35) and a nucleotide sequence encoding a fusion protein optionally with a protective peptide added to the desired peptide via a

linker sequence, and collecting the desired peptide or the fusion protein from the culture;

step (2); a step of cleaving and separating the protected peptide and, optionally, the linker sequence and the desired peptide from the resulting fusion protein, and optionally further purifying the desired peptide when the fusion protein is collected in the step (1); and

step (3); a step of protecting, with a protecting group, one or more reactive functional groups which may cause an undesirable side reaction, selected from the group consisting of a hydroxy group, an amino group, a guanidino group, an imidazolyl group, an indolyl group, a mercapto group and a carboxyl group, in the side chain of the desired peptide obtained in the step (1) or the step (2).

Claim 36 (new): The method for producing a modified peptide or protein according to claim 35, wherein cleavage and separation of the protective peptide and, optionally, the linker sequence and the desired peptide in the step (2) is performed at two steps using an OmpT protease or a derivative thereof and Kex2 protease or a derivative thereof.

Claim 37 (new): The method for producing a modified peptide or protein according to claim 35, wherein the linker sequence is a sequence set forth in SEQ ID NO: 27.

Claim 38 (new): The method for producing a modified peptide or protein according to claim 34, wherein the peptide fragment is a peptide fragment containing no modified amino acid or non-amino acid in ghrelin or a derivative thereof.

Claim 39 (new): The method for producing a modified peptide or protein according to claim 34, wherein the protected peptide fragment containing no modified amino acid or non-amino acid is purified and stored in a solution having a pH of 4 to 8.

Claim 40 (new): The method for producing a modified peptide or protein according to claim 34, wherein the protecting group is a Boc group.

Claim 41 (new): A method for producing a protected peptide fragment containing no modified amino acid or a non-amino acid, which comprises producing the peptide fragment by a

method comprising:

step (1); a step of culturing a cell transformed with an expression vector having one of a nucleotide sequence encoding a peptide having the desired amino acid sequence (hereinafter, referred to as desired peptide, in the present claim 41) and a nucleotide sequence encoding a fusion protein optionally with a protective peptide added to the desired peptide via a linker sequence, and collecting the desired peptide or the fusion protein from the culture;

step (2); a step of cleaving and separating the protective peptide and, optionally, the linker sequence and the desired peptide from the resulting fusion protein and, optionally further purifying this, when the fusion protein is collected in the step (1);

step (3); a step of protecting, with a protecting group, one or more reactive substituents which may cause an undesirable side reaction, selected from the group consisting of a hydroxy group, an amino group, a guanidino group, an imidazolyl group, an indolyl group, a mercapto group and a carboxyl group, in the side chain of the desired peptide obtained in the step (1) or (2); and

step (4); a step of purifying and storing the protected desired peptide obtained in the step (3) in a solution having a pH of 4 to 8.

Claim 42 (new): The method for producing a protected peptide fragment containing no modified amino acid or non-amino acid according to claim 41, wherein the protecting group is a Boc group.

Claim 43 (new): The method for producing a protected peptide fragment containing no modified amino acid or non-amino acid according to any one of claims 41 or 42, wherein cleavage and separation of the protective peptide and, optionally, the linker sequence and the desired peptide in the step (2) is performed at two steps using an OmpT protease or a derivative thereof and Kex2 protease or a derivative thereof.

Claim 44 (new): The method for producing a protected peptide fragment containing no modified amino acid or non-amino acid according to any one of claims 41 or 42, wherein the linker sequence is a sequence set forth in SEQ ID NO: 27.

Claim 45 (new): The method for producing a modified peptide or protein according to any one of claims 41 or 42, wherein the peptide fragment is a peptide fragment containing no modified amino acid or non-amino acid in ghrelin or a derivative thereof.

Claim 46 (new): The method for producing a peptide fragment according to claim 30, wherein a protecting group for a reactive functional group in a side chain of an amino acid or a non-amino acid A which is to be modified with substituent R is a silyl protecting group, and a quaternary ammonium fluoride is used for eliminating the protecting group.

Claim 47 (new): The method for producing a peptide fragment according to claim 46, wherein the silyl protecting group is t-butyldimethylsilyl (TBDMS), t-butyldiphenylsilyl (TBDPS), triisopropylsilyl (TIPS), triisobutylsilyl (TIBS), t-hexyldimethylsilyl (ThxDMS) or triphenylsilyl (TPS), and the quaternary ammonium fluoride is tetrabutylammonium fluoride (TBAF), tetraethylammonium fluoride (TEF) or ammonium fluoride.

Claim 48 (new): The method for producing a peptide fragment according to any one of claims 30, 46, or 47, wherein A is serine, threonine, cysteine, homocysteine, lysine, ornithine, glutamic acid, 2-aminoadipic acid, diaminoacetic acid, 2-aminomalonic acid, aspartic acid, tyrosine or asparagine, and R is bound to a reactive substituent in the side chain of A via an ester bond, an ether bond, a thioether bond, a disulfide bond, an amide bond, an O-glycoside bond or an N-glycoside bond.

Claim 49 (new): The method for producing a peptide fragment according to claim 48, wherein A is serine or threonine, and R is bound to the hydroxy group in the side chain of A via an ester bond.

Claim 50 (new): The method for producing a peptide fragment according to claim 49, wherein the peptide fragment is ghrelin or a derivative thereof, or a peptide fragment containing a modified amino acid in the ghrelin or a derivative thereof.

Claim 51 (new): The method for producing a modified peptide or protein according to claim 36, wherein the linker sequence is a sequence set forth in SEQ ID NO: 27.